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# Development and Validation of Stability Indicating Hptlc Method for Estimation of Stiripentol

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**ABSTRACT**: Stiripentol is an antiepileptic drug that is used for the treatment of Dravet syndrome. A stability-indicating HPTLC method has been established & validated for the determination of pestilential. The stationary phase used was Silica Gel plate 60F254 &mobile phase was selected astoluene: ethyl acetate (9.5:0.5 V/V). The Rf was found to be (0.46 ± 0.02). The developed stability-indicating method was validated for linearity, accuracy, precision,limitofdetection,limitofquantitation,and robustness parameters after establishing stability by forced degradation study. Stiripentol wasfound tobe sensitivetoacidicaswellasalkalinehydrolyticstress, and oxidativeandthermal degradation conditionsbut nopeakfor degradationproduct wasdetectedin spite of multiple wavelength scanning.

**KEYWORDS:** Stiripentol, High-Performance ThinLayerChromatography,forceddegradation, validation, Stability-indicating method.

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## I. INTRODUCTION

Chemically, pestilential is (1E)-1-(1,3-benzodioxol-5-yl)-4,4-dimethyl-pent-1-en-3-ol[14]. It is soluble in methanol and practically insoluble in water at room temperature. Stiripentol, (Diacomit; Biocodex Inc.) has been approved by the European and Canadian marketing authorization for the treatment of Dravet syndrome as adjunctive therapy with clobazam and valproic acidnot onlyin children but also during adolescence and adulthood when seizures are not adequately controlled with the association of these two medications[1,2,4,18]. Dravet syndromeis also called severe myoclonic epilepsy of infancywhichisprogressiveepileptic encephalopathy[10,13,19]. Because of the inhibitory effect of pestilential on hepatic cytochrome-P450, its clinical development was delayed[6]. Its principal mechanism ofaction inthe brain isGABA inhibitory neurotransmission and by blocking the GABA- transaminase activity it prevents the GABA metabolism[16]. The literature search shows that there arereports ofbioanalytical High-Performance Liquid Chromatography (HPLC) method[12,15,17], "stability indicatingmethod" byHPLC[3]and HPTLC[11]. After lookingattheliteraturesurvey,itwasconcluded thatthere was work done on stability indicating method by HPTLC & HPLC method, but it is observed thatthereis a scopetooptimizetheHPTLC methodintermsofthelevel ofstressdegradation.So, in this work by enhancing the stress condition parameterslike strength of stress reagent and time of exposure, the method for stability study was well optimized. Hence the main aim of our study was to optimize the various stress conditions for pestilential and evaluate the optimized HPTLC method.

## II. MATERIALSANDMETHODS

### Materials

Thedrugpestilentialwasreceivedasagiftsample. All the chemicals used were of AR grade viz. methanol, toluene, ethyl acetate, dichloromethane, hydrochloric acid, sodium hydroxide, hydrogen peroxide.

### Methods Instruments:

UV-spectral analysis was performed on UV- Visible spectrophotometer (Make-JASCO, Model-V730).CAMAGHPTLCsystemequippedwitha Linomat 5 sample applicator operated under a gentle stream of nitrogen, coupled with a Hamilton microliter syringe (100 µL) was used for application purpose. CAMAG TLC SCANNER 3

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was used for detectionanddensitometryscanning.Dataacquisition was done by WINCATS software (version 1.4.3). Photostabilitystudywasperformedinaphotostability chamber (Make- NEWTRONIC, Model-NEC103RSPI).

**Detectionofwavelength**

A solution of pestilential (10 µg $mL^{-1}$) was prepared using methanol and UV spectrum was recorded. Itshowedmaximumabsorbanceat262nm. Spectrum is shown in (Fig.1)

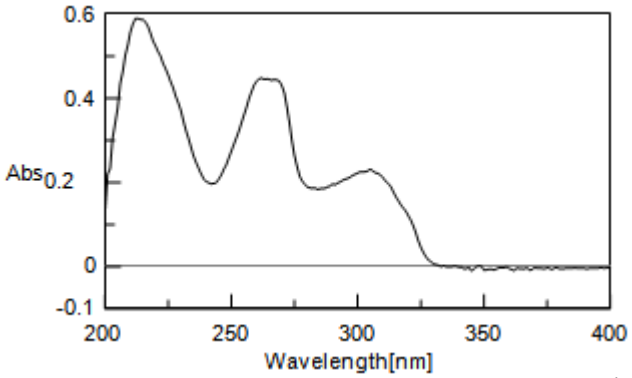


**Fig.1:UVspectrumofpestilentialinmethanol(10 µg $mL^{-1}$)**

## III. PREPARATION OF STANDARD AND SAMPLE SOLUTION

For thepreparation of standard solution, 10mg of thedrugwasaccuratelyweighedandtransferredtothe volumetricflaskof10mLcapacity. Then added some methanol and made up the volume after the drug was dissolved to obtain the solution of 1000 µg $mL^{-1}$.A solution of strength 50µg $mL^{-1}$was prepared by appropriatedilution&wasusedasaworkingsolution. Stiripentol capsules and sachets are available as Diacomit in 250 & 500 mg strength. But the brand Diacomitwasnotavailableinlocalmarket.Hence,we preparedanexcipientblendtowhichAPIwasspiked. Forthepreparationspikedblend,125mgstarch&125 mglactoseweremixedinthemortarpestle.Then250 mgofpestilentialwasmixedwiththeaboveexcipients. Fromthisspikedblend,20mgofblend(equivalentto 10mgofdrug)wasaccuratelyweighedanddilutedto 10 mL with methanol to obtain a solution (1000 µg $mL^{-1}$). The solution was sonicated & then filtered using Whatman filter paper. It was diluted to get 50 µg $mL^{-1}$ working solution.

### Chromatographic Conditions

Chromatographic separation of pestilential drug was performed on Aluminium plates precoated with a band of 6 mm width using a 100µL syringe with a Linomat applicator.The mobile phase was composed oftoluene: ethyl acetate(9.5:0.5V/V). Atwintrough glass chamber (10 cm × 10 cm)was used for linear ascendingdevelopment of theTLC platewith 15min saturation time, migration distance was 70 mm. Densitometric scanning was performed at 262 nm, operatedbysoftware,slitdimensionswere4x0.45 mm. The standard densitogram of pestilential (250ng $band^{-1}$) is shown in (Fig. 2).

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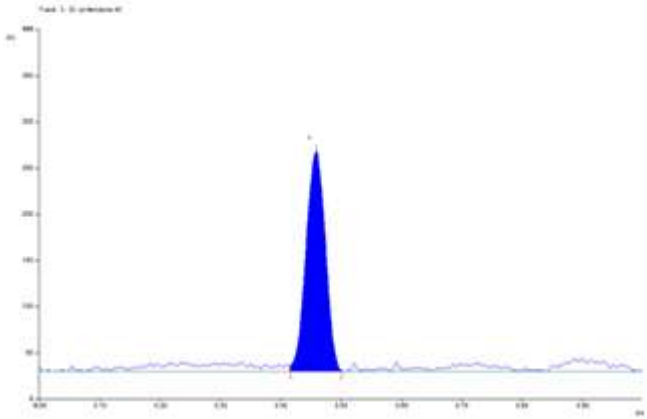

**Fig.2:Representativedensitogramofpestilential (250 ng band$^{-1}$, Rf = 0.46 ± 0.02)**

**Calibrationcurvepreparation**

Therangeselectedwas250-1250ngband$^{-1}$to silicagel60$F_{254}$.Sampleswereappliedontheplateas determine the linear relationship between peak area and amount spotted.

## IV. FORCEDDEGRADATIONSTUDY

Various stress conditions have been applied to check the degradation[7]. The conditions were hydrolysis under various pH viz. acid, alkali conditions; oxidative, thermal stress, photostability[9]under UV and fluorescent light. The conditions were optimized to achieve degradation in the range of 10- 30 %.

**AcidHydrolysis:**

For sample preparation, 1 mL of stock solution (500 µg mL$^{-1}$) was mixed with 1mL 0.05NHCL and thevolumewasmadeupto10mLwithmethanol.The solutionwaskeptfor20minatroomtemperature.The resultant solution of 50µg mL$^{-1}$ was applied to the TLC plateandthedensitogram was shown in (Fig 3).

**AlkaliHydrolysis**

For sample preparation, 1 mL of stock solution (500 µg mL$^{-1}$) was mixed with 1 mL 1N NaOH, and thevolumewasmadeupto10mLwithmethanol.The solutionwaskeptfor1hatroomtemperature.The resultantsolutionof50µgmL$^{-1}$wasappliedtothe TLC plate.

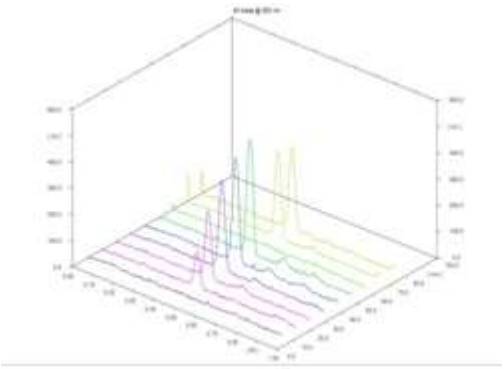

**Fig.3:3Ddensitogramofacidhydrolysis (Track 1 methanol blank; track 2-6 standard linearity; track 7 acid blank;track8,9aciddegradation)**

**OxidativeDegradation**

For sample preparation, 1mL of stock solution (500 µg mL$^{-1}$) was transferred to a volumetric flask then 1mL of 3% V/V $H_2O_2$was added and volume made up to 10mL with methanol and was kept for 30minatroomtemperature.Theresultantsolutionof 50 µg mL$^{-1}$was applied to the TLC plate.

**ThermalDegradation**

Bulk drug powder was exposed to 50°C temperaturein ahot air oven for 1h.Thesamplewas

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cooledtoroomtemperatureandthen10mgofpowder was accuratelyweighedanddissolved in methanol to 10mL.Asolutionofstrength 50µgmL$^{-1}$wasprepared byappropriate dilution and applied to the TLC plate.

**PhotolyticDegradation**

The sample was exposed to UV light for not less than200 WH m$^{-2}$andwhitefluorescentlightofillumination fornotlessthan 1.2millionluxh.Afterexposure, 10 mg of powder was accuratelyweighed and dissolved in methanol to 10 mL.Asolutionofstrength50µgmL$^{-1}$waspreparedby appropriate dilution and applied to the TLC plate.

**Methodvalidation**[8]

Themethod wasvalidated for theparameterslike linearity,precision,accuracy,assay,robustness,LOD, LOQ.

**Linearity**

Fromtheworkingstocksolution ofstrength50µg mL$^{-1}$,appropriate volumeslike 5,10,15,20,25µL were spotted on the TLC plate to obtain the range of 250- 1250 ng band$^{-1}$.

**Precision**

Repeatability and intermediate precision were studied by spotting six replicates of the lowest concentration from the linearity range i.e. 250 ng band$^{-1}$ on the same day & three consecutive days.

**AssayandAccuracy**

Forassay,thesamplerequiredi.e. 50µgmL$^{-1}$was prepared from the 1000 µg mL$^{-1}$sample solution. For the accuracy study, the standard addition method was used at 80%, 100%, and 120%.

**Limit of Detection and Limit of Quantitation (LOD and LOQ)**

The detection and quantitation limit were calculated from calibration curve. Both were calculated using the formulae, LOD = 3.3 SD/S and LOQ = 10 SD/S, where SD is the standard deviation ofthelowestresponse;Sistheslopeofthecalibration curve.

**Robustness**

Five parameters were changed deliberately and slightly i.e. time from scanning to development, time ofdevelopmentfromapplication,wavelength,mobile phase ratio, chamber saturation time.

## V. RESULTS AND DISCUSSION

**ForcedDegradationStudies**

Inordertoevaluatethestabilityindicatingpro perty of the developed method, forced degradation studies were carried out and optimized to 10-30 % degradation in accordance with International Conference on Harmonization (ICH) guidelines Q1A (R2). See (Table I).Stiripentol was found to be sensitivetoacidicaswellasalkalinehydrolyticstress, oxidative and thermal degradation conditionsbut no peakfor degradation productwasdetected,even after multiplewavelengthscanningwhichisshownin(Fig. 4)

| Sr. no. | Stresscondition | Concentration and time | % recovery |
|---|---|---|---|
| 1 | Acidhydrolysis | 0.05NHClfor20 min | 76.23 |
| 2 | Alkaline hydrolysis | 1NNaOHfor1h | 75.87 |
| 3 | Oxidative degradation | 3%$H_2O_2$for30 min. | 74.56 |
| 4 | Thermal Degradation | 50ºCfor1h | 87.36 |
| 5 | UVdegradation | 200 WHm$^{-2}$ | 96.71 |
| 6 | Fluorescence degradation | 1.2million luxh | 93.84 |

**TableI:Summaryofforceddegradationstudy**

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**Method validation**

**LinearityandRange**

The range selected was 250-1250 ng band$^{-1}$ and the correlation coefficient were found 0.99 with equation of y= 7.319x+ 1765.7. The densitogram of linearityandresidualplotareshowninthe(Fig.5)and (Fig. 6) respectively.

The linear relationship between amount spotted and peak area is confirmed by residual plot.

This residual plot without any tendency proves the linearity of calibration[5].

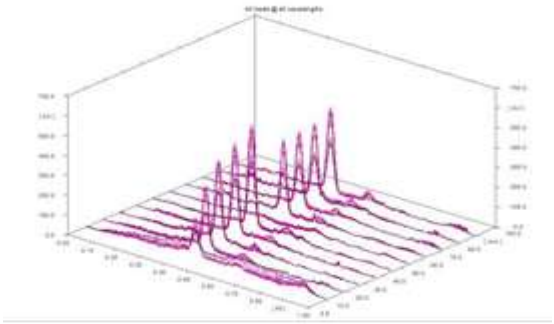

**Fig.4:3Ddensitogramofmultiplewavelength scanning**

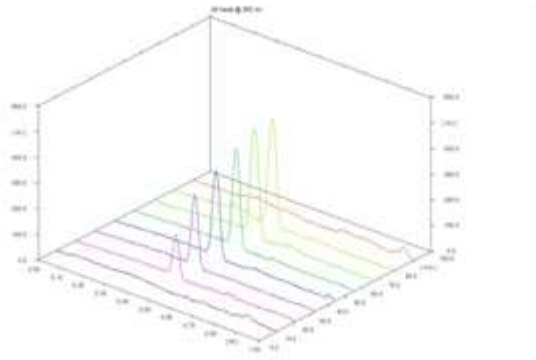

**Fig.5:3Ddensitogramofpestilentiallinearity (250-1250 ng band$^{-1}$)**

**AssayandAccuracy**

Assaywasfoundtobe100.67%andaccuracy result was shown in (Table II) and (Fig.7).

**Precision**

RepeatabilityandIntermediateprecisionwere performed and densitogram was shown in (Fig. 8). %RSDforwasfoundtobe0.28%&1.25% respectively.

**Limitofdetection(LOD)andlimitof quantitation (LOQ)**

LODandLOQwerecalculatedbyy-intercept method.LODandLOQwasfoundtobeinrangei.e., 10.89and33.01ngband$^{-1}$respectively.

| Sr.No | Amountfrom marketed formulation (ng band$^{-1}$) | Amount of standardadded (ng band$^{-1}$) | Totalamountof the drug (ngband$^{-1}$) | % recovery |
|---|---|---|---|---|
| 1 | 500 | 400 | 900 | 101.34 |
| 2 | 500 | 500 | 1000 | 101.72 |
| 3 | 500 | 600 | 1100 | 100.54 |

**TableII:%recovery(accuracy)studies**

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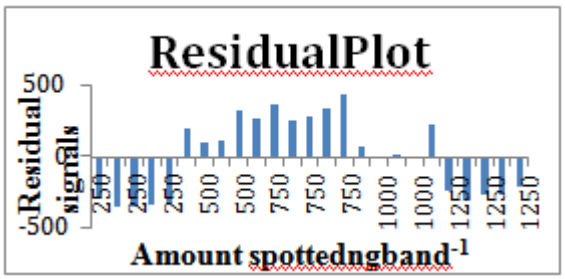


**Fig.6:Residualplot**

**Limitofdetection(LOD)andlimitof quantitation (LOQ)**
LODandLOQwerecalculatedbyy-intercept method.LODandLOQwasfoundtobeinrangei.e., 10.89and33.01ngband$^{-1}$respectively.

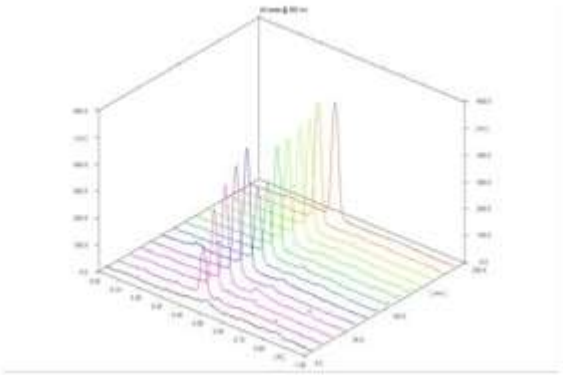

**Fig.7:3Ddensitogramofaccuracy(%recovery) (Track 1 methanol blank; track 2-6-linearity, track7,8-assay,track9,10-standardaddition80 %,track11,12-standardaddition100%,track 13,14-standard addition 120 %)**

**Robustness**

It wasobservedthat %relativestandarddeviation (% RSD) NMT 1.5 %, which confirmed that the developed method was robust. For results of robustness see (Table III).

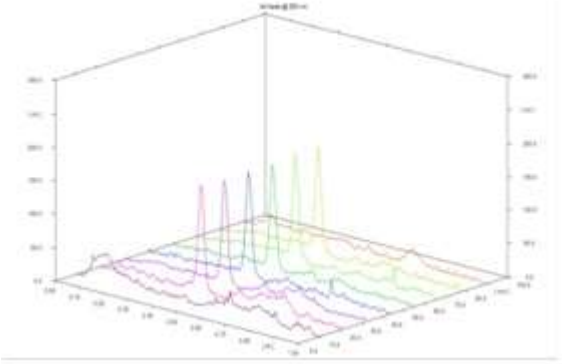

**Fig.8:3DDensitogramofprecision(250ngband$^{-1}$)**

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## VI. DISCUSSION

The stability-indicating HPTLC method for pestilentialwasdeveloped&validated.Incomparison totheresultsreportedbyKashidS.et.al,ourproposed method has an advantage of being a binary mobile phase instead of ternary. The stress conditions for degradation were optimized to obtain degradation in the range of 10-30 % as per ICH guidelines. To the contrary, in Kashid S. et.al. study, the reported % degradation values are < 10 %. Our work is simple, well optimized & reproducible. The application of forced degradation by the proposed method could be used for monitoring extent of degradation.

| **Parameter** | **Condition** | **%RSD** |
|---|---|---|
| Mobile phase composition Toulene: ethyl acetate(9.5:0.5 V/V) | 9.4:0.6V/V | 0.96 |
| | 9.6:0.4V/V | 1.15 |
| Saturationtime(15 ±5min) | 10min | 0.89 |
| | 20min | 1.68 |
| Time from applicationto development | Immediate | 1.16 |
| | After2h | 1.16 |
| Time from developmentto scanning | Immediate | 1.27 |
| | After2h | 1.27 |
| Changein wavelength(262±2 nm) | 260nm | 1.26 |
| | 264nm | 1.00 |

**TableIII:Robustnessstudies**

## VII. CONCLUSION

Thedeveloped HPTLCmethodfor estimation of pestilentialwasfoundtobeaccurate,precise,specific. The method was validated as per guidelines of ICH Q2R1.Stiripentol was found to be sensitive to acidic as well as alkaline hydrolytic and oxidative and thermal degradation conditions but no degradation product peak was detected. The developed chromatographic method may be used for routine analysis of pestilential.

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